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TITLE: Overcoming the Practical Barriers to Spinal Cord Cell Transplantation for ALS

PRINCIPAL INVESTIGATOR: Nicholas Boulis

CONTRACTING ORGANIZATION: Emory University , Atlanta, GA 30322

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This grant has provided so far critical data on tolerance and toxicity of cell dosing and numbers of permissible spinal cord injections. Rigorous experiments in Aim 1 of our grant have demonstrated that, even though the porcine spinal cord seems to tolerate escalating numbers and volumes of injections, the severity of acute transient morbidity should not be neglected. Moreover, escalating numbers and volumes of injections seem to be associated with lack of accuracy and reflux.
At the same time, little is understood about the appropriate immunosuppressive therapy for spinal cord stem cell transplant recipients. In our ongoing human trial, aggressive immunosuppressant therapy has formed the single biggest source for adverse events. Aim 2 will help us to optimize immunosuppression, preventing needless complications.

15. SUBJECT TERMS

ALS, spinal cord, stem cell, swine, therapy, transplantation.

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INTRODUCTION:

"The present application refines critical details required for successful cell transplantation. Aim 1 (Optimal Surgical Technique) has provided critical data on tolerance and toxicity of cell dosing and numbers of permissible spinal cord injections. Aim 2 (Graft Rejection) will provide critical data on graft rejection and appropriate immunosuppression for human spinal cord stem cell transplantation."

KEYWORDS: ALS, cell therapy, escalation, graft, number, pigs, safety, spinal cord, swine, tolerance, transplantation, volume.

ACCOMPLISHMENTS:

In Year 3 we have completed histological and stereological analyses pertaining to Aim 1.
We are currently performing surgical procedures pertaining to Aims 2c and 2d.
A total of 25 animals have been / are planned to be used.

	Year 1	Year 2	Year 3	Year 4*
Aim 1				
Produce cells	XXXXXXXXXX			
a. Injection #	XXXXXXXXXX	XXXXXXXXXX	XX	
b. Volume	XXXXXXXXXX	XXXXXXXXXX	XX	
c. Platform vs. Hand-held	XXXXXXXXXX	XXXXXXXXXX	XX	
Aim 2				
Produce cells		XXXXXXXXXX		
a. Allo vs. Xeno		XXXXXXXXXX	XXXX	XX
b. Triple vs. Monotherapy		XXXXXXXXXX	XXXX	XX
c. Repeated injections = Sensitization			XXXXXXXXXX	XXXX
d. Immunosuppression withdrawal			XXXXXXXXXX	XXXX

** An Extension With-Out Funds (EWOF) has been requested to finalize Aim 2.*

Detailed Aim 2

Explanation	Pigs	# Injections	Cell Type	Immunosuppression	Survival (days)
a. Allo vs. Xeno	5	10	Pig NPCs	None	21
	5			Tacrolimus	
	5		Human NPCs	None Tacrolimus	
	5				
b. Triple vs. Monotherapy	5	10	Pig NPCs	Basiliximab + MMF + Tacrolimus	21
c. Repeated Injections = Sensitization*	5	10 + 10	Pig NPCs + Pig NPCs	Tacrolimus	21 + 21
	5	10 + 10	Vehicle + Pig NPCs		
d. Immunosuppression Withdrawal*	5	10	Pig NPCs	None	42
	5			Tacrolimus for 14 days	
	5			Tacrolimus for 42 days	

Ongoing

** Peripheral blood (serum) has been collected pre-op and every 7 days until endpoint for the following analyses:*

- Development of graft-specific pig antibodies with a Flow Cytometry Cross Match (FCXM)

- Detection of graft-specific pig lymphocytes with a modified Mixed Lymphocyte Reaction (MLR)

IMPACT:

Data generated in Aim 1 has been **"**disseminated to communities of interest" and consequently assisted other research groups (Svendsen group, Q Therapeutics) in their planning for upcoming trials and IND submissions to the FDA for ALS indication.

CHANGES/PROBLEMS:

On Feb 2014, we received approval for a rebudgeting / termination of subcontract (see **Appendix 1**). The former Subcontract (Svendsen laboratory at Cedar Sinai) could not fulfill their original plan of delivering porcine iPS-derived NPCs or fetal cortical-derived pig NPCs. As an alternative, we established collaboration / agreement with Neuralstem, Inc. to utilize their GFP-expressing fetal spinal cord-derived pig NPCs to complete Aims 2c and 2d.

PRODUCTS:

Reportable outcome

Abstracts

1. Gutierrez J, Lamanna JJ, Grin N, Hurtig CV, Miller JH, Riley J, Urquia L, Federici T, Boulis NM. Preclinical Validation of Multilevel Intraspinal Stem Cell Therapy for Amyotrophic Lateral Sclerosis (ALS). Congress of Neurological Surgeons Annual Meeting (Boston, October 2014).

2. Lamanna JJ, Gutierrez J, Espinosa JR, Urquia L, Grin J, Hurtig CV, Riley J, Bordeau J, Polak M, Brannon P, Glass J, Federici T, Kirk AD, Boulis NM. Peripheral Monitoring of Immune Response to Intraspinal Stem Cell Therapy. Congress of Neurological Surgeons Annual Meeting (Boston, October 2014). – **Appendix 2**

Manuscripts

1. Gutierrez J, Lamanna JJ, Grin N, Hurtig CV, Miller JH, Riley J, Urquia L, Svendsen CN, Federici T, Boulis NM. Preclinical Validation of Multilevel Intraspinal Stem Cell Therapy for Amyotrophic Lateral Sclerosis (ALS). *Submitted*. **Appendix 3**

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name:	Nicholas Boulis, MD
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.36
Contribution to Project:	Principal Investigator
Funding Support:	DoD - 3% Others (Departmental, Restricted Research, and Restricted Gift) 97%

Name:	Thais Buchman, PhD
Project Role:	Key Personnel – Project Manager
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	3
Contribution to Project:	Project Manager / Histology
Funding Support:	DoD – 25% Restricted Research – NIH 10% Restricted Gift 65%

Name:	Natalia Grin (former employee)
Project Role:	Veterinary Technician
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	6
Contribution to Project:	Surgical Assistant / Animal Treatment and Care / Histology
Funding Support:	DoD – 50% Restricted Research – ALS Association 50%

Name:	Juanmarco Gutierrez, MD
Project Role:	MS Student
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	12
Contribution to Project:	Surgeon / Stereology / Analysis / Manuscript Author
Funding Support:	DoD

Name:	Lindsey Urquia
Project Role:	Laboratory Assistant, Senior
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	12
Contribution to Project:	Surgical Assistant / Animal Treatment and Care / Histology
Funding Support:	DoD

Name:	Jason Lamanna
Project Role:	MD/PhD Student
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	6
Contribution to Project:	Cell preparation / Surgeon / Immunology
Funding Support:	Restricted Research – ALS Association 35% Restricted Gift 35% Restricted Research 30%

Name:	Cheryl Moreton
Project Role:	Volunteer
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	3
Contribution to Project:	Histology / Stereology
Funding Support:	n/a

Partner Organizations:

- Organization Name: Neuralstem, Inc.
- Location of Organization: 20271 Goldenrod Lane - Germantown, MD - 20876
- Partner's contribution to the project
    - Financial support: none
    - In-kind support: cells for Aims 2c and 2d
    - Facilities: none
    - Collaboration: none
    - Personnel exchanges: none
    - Other.

SPECIAL REPORTING REQUIREMENTS:

N/A.

APPENDICES:

Appendix 1 – Abstract - Immunology Data

Appendix 2 – Summary of Aim 1 Data for Manuscript Submission

Appendix 1 – Abstract - Immunology Data

Peripheral Monitoring of Immune Response to Intraspinal Stem Cell Therapy

Lamanna JJ[1,2], Gutierrez J[1], Espinosa JR[3], Urquia L[1], Grin N[1], Hurtig CV[1], Polak M[4], Bordeau J[4], Brannon P[5], Glass JD[4], Kirk AD[3], Federici T[1], and Boulis NM[1,2].

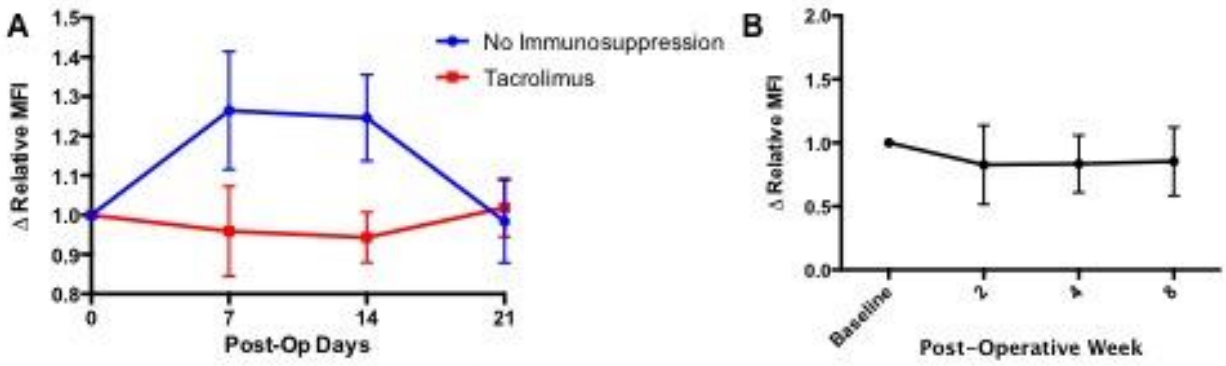
1. Department of Neurosurgery, Emory University, Atlanta, GA
2. Department of Biomedical Engineering, Emory University & Georgia Institute of Technology, Atlanta, GA
3. Department of Surgery, Emory University, Atlanta, GA
4. Department of Neurology, Emory University, Atlanta, GA
5. Histocompatibility and Cytogenetics Laboratory, Emory University Hospital, Atlanta, GA

Introduction: Clinical investigations of intraspinal stem cell therapies are underway for a range of neurological diseases. Originally considered entirely immuno-privileged, the CNS is now considered relatively privileged and immunological reactions to exogenously transplanted cell grafts have been demonstrated in mammalian models. From these observations, immunosuppression regimens have been employed clinically. However, graft rejection remains a significant risk and an assay to non-invasively monitor the immune response to transplanted intraspinal cell grafts is essential. We hypothesize that graft-specific host antibodies may be detected in the peripheral blood.

Methods: Ten minipigs received five intraspinal injections of 100,000 donor human neural progenitor cells. Five pigs received tacrolimus and five received no immunosuppression. Plasma was isolated from peripheral blood collected pre-transplant and serially post-transplant. Furthermore, plasma was collected from six patients with Amyotrophic Lateral Sclerosis enrolled in the Phase 1 trial at Emory (NCT01348451) receiving intraspinal injections of donor human neural stem cells. The patients received tacrolimus, mycophenolate mofetil, and basiliximab. Plasma was collected from three patients with naïve transplants and three patients with second transplants. Donor stem cells were incubated with collected plasma and then incubated with a fluorescent conjugated antibody specific to either pig or human IgG to measure the presence of graft-specific antibodies in the plasma. Relative mean fluorescent intensity (MFI) was measured with flow cytometry and compared to pre-operative baseline.

Results: A transient increase in graft-specific antibodies was detected one and two weeks post-operatively in pigs that did not receive immunosuppression. No increase was observed in the tacrolimus group. In patients, a trend showing a slight decrease in graft-specific antibodies was observed.

Conclusions: This method can be used to detect antibody-mediated graft rejection *in vivo*. Furthermore, it provides evidence for a decreased immune response to transplanted intraspinal stem cell grafts with immunosuppression.



In Vivo Detection of Graft-Specific Antibodies. Flow cytometry calculated relative change of mean fluorescent intensity (Δ Relative MFI) data from pig (**A**) and human (**B**) plasma following intraspinal stem cell transplantation. Δ Relative MFI is calculated by normalizing post-operative measurements to the pre-operative, baseline measurement. Error bars: ±SEM.

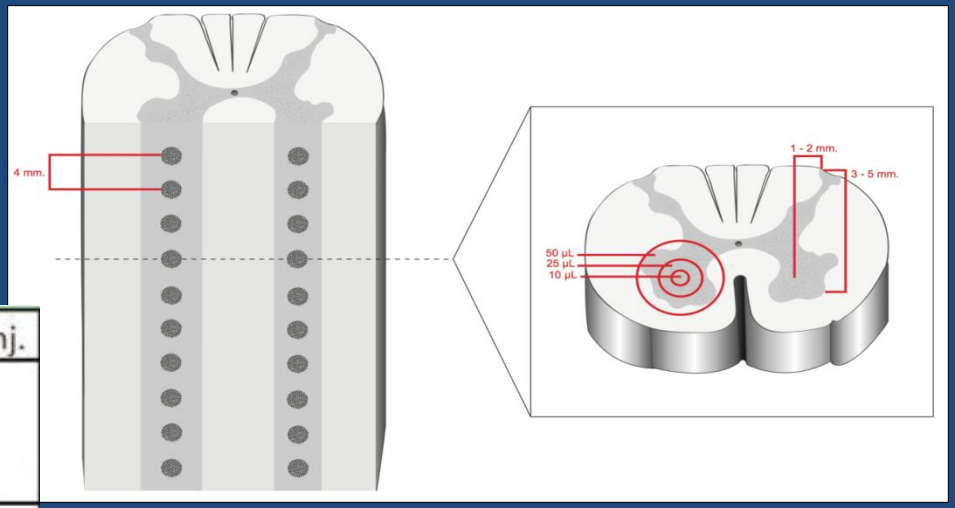
Appendix 2– Summary of Aim 1 Data for Manuscript Submission

Aim 1 (**Optimal Surgical Technique**) will provide critical data on tolerance and toxicity of cell dosing and numbers of permissible spinal cord injections.

- Number-escalation: 20, 30, and 40 injections

- Volume-escalation: 10, 25, 50, (and 75) microliters / injection

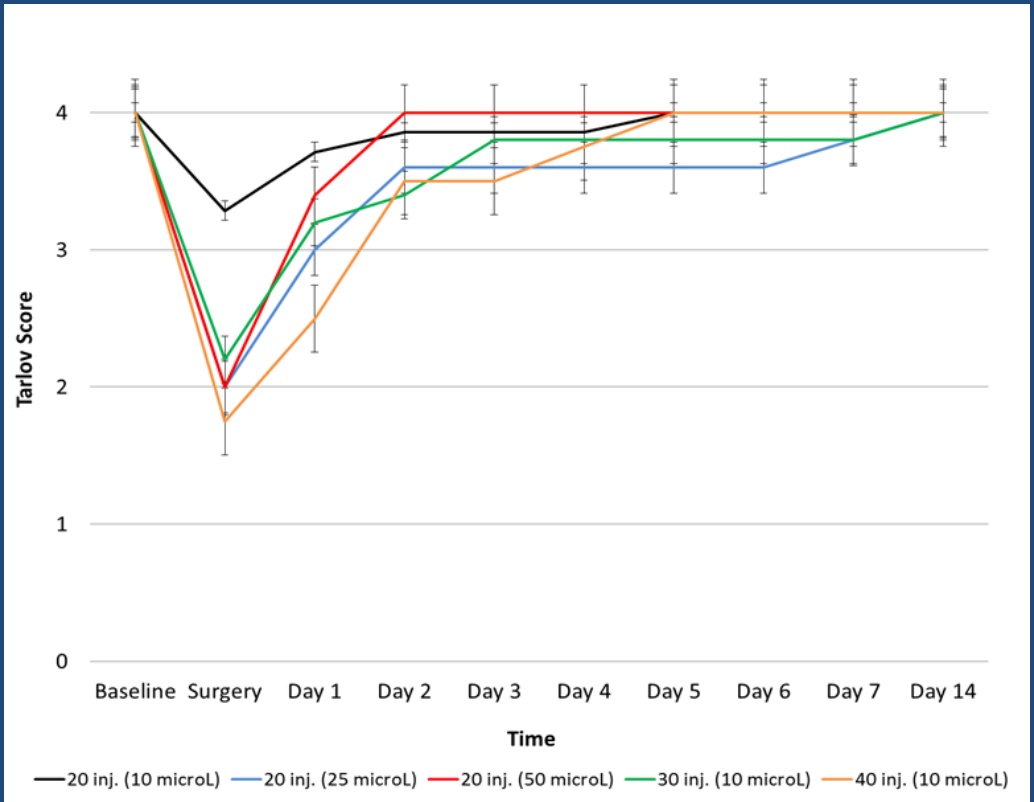
- Platform/Floating Cannula vs. Hand-held injections



Injection Site	Side	Cannula Type	Number Inj.	Volume Inj.
Cervical	Bilateral	Floating	20	10
			30	
			40	
Cervical	Bilateral	Floating	20	10
				25
				50
				75
Cervical	Bilateral	Hand-held	20	10
				25
				50

Aim 1a **- There is a *number of injections* that forms a threshold for permanent neurological morbidity.** Dose-escalation of 20, 30, and 40 bilateral cervical injections of 10ul/each.

Aim 1b **- There is a threshold for morbidity in terms of *volume of injections*.** Dose-escalation of volume (10ul, 25ul, 50ul, and 75ul / injection, with a total of 20 bilateral cervical injections)



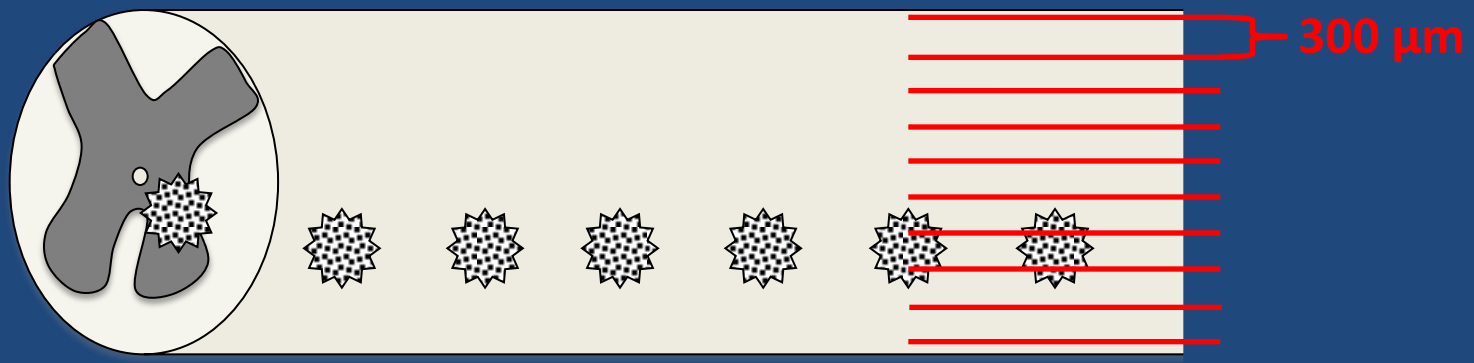
Behavioral Outcome

- Transient morbidity but ALL ANIMALS come back to baseline motor function

	Volume escalation (20 injections)			Number escalation (10 µL)	
Outcome measure	10 µL	25 µL	50 µL	30 injections	40 injections
Time back to baseline Tarlov score (days)	1.14 ± 1.77	2.8 ± 2.95	1.40 ± 0.55	2.8 ± 3.03	3.25 ± 1.50
*** Note: values reported are mean ± standard deviation**					

Aim 1 - Histological Outcome / Quantifications

- Neuron number per mm^3
- Number of damaged injection sites
- Migration patterns
- Engraftment percentage



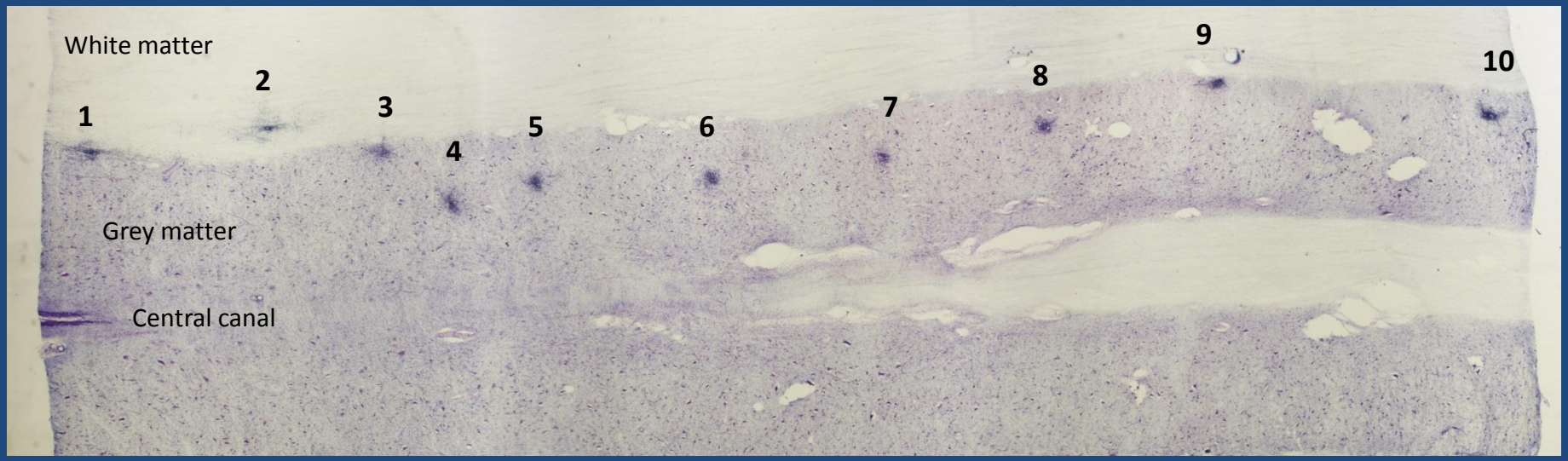
* Section thickness → 40 – 50 µm

1 out of every 6 sections is sampled for **stereological analysis**

↓

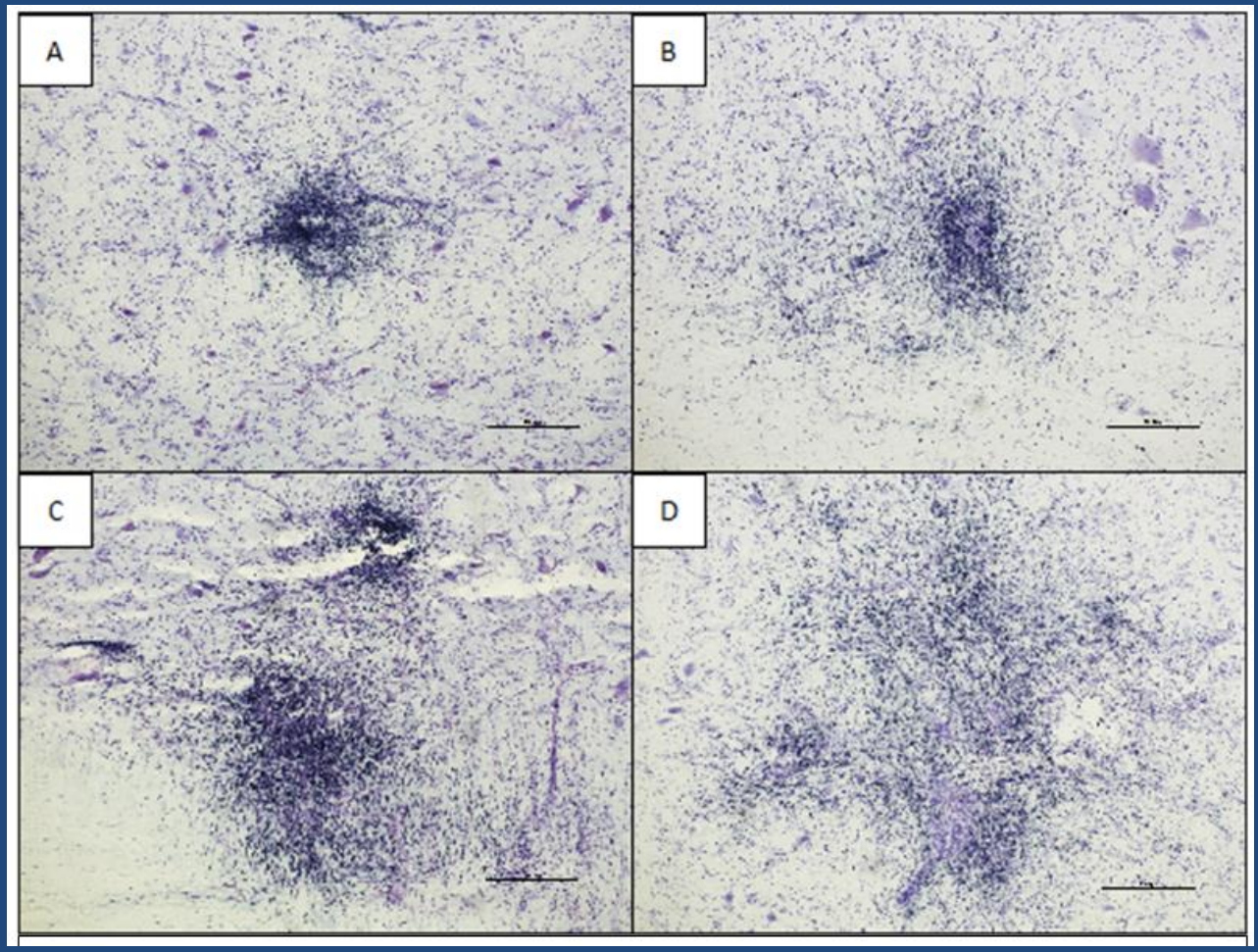
Neurons and transplanted cells counted using unbiased methods
(Cavalieri principle and Optical Disector)

Histological Outcome - Number Escalation

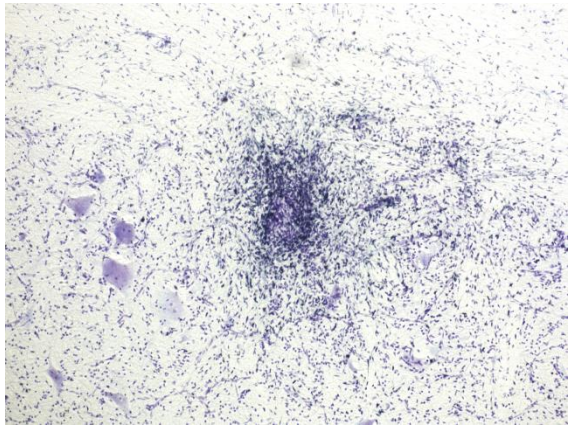


	Volume escalation (20 injections)			Number escalation (10 µL)	
Outcome measure	10 µL	25 µL	50 µL	30 injections	40 injections
Percentage of identified grafts	61.71 ± 39.73	64 ± 13.87	60 ± 10.61	70.67 ± 10.65	79.375 ± 14.05
Number of identified grafts	12.14 ± 7.95	12.8 ± 2.77	12 ± 2.12	21.2 ± 3.19	31.75 ± 5.62
Engraftment percentage	21.04 ± 14.89	11.74 ± 5.82	12.01 ± 12.07	31.07 ± 17.14	18.2 ± 7
Total number of grafted cells	343,805 ± 328,064	393,822 ± 264,216	670,832 ± 581,591	667,972 ± 401,573	573,746.5 ± 202,435
*** Note: values reported are mean ± standard deviation**					

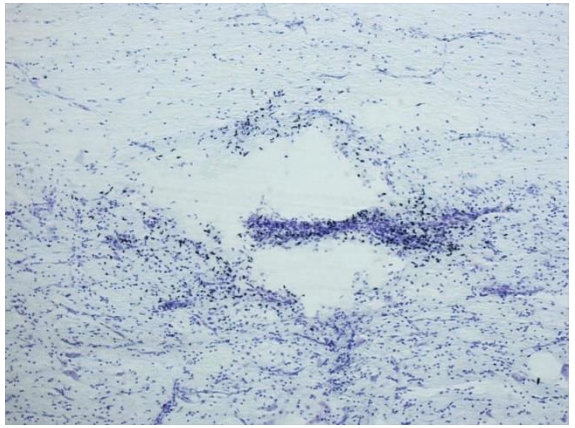
Histological Outcome - Volume Escalation



Histological Outcome – Damaged Sites



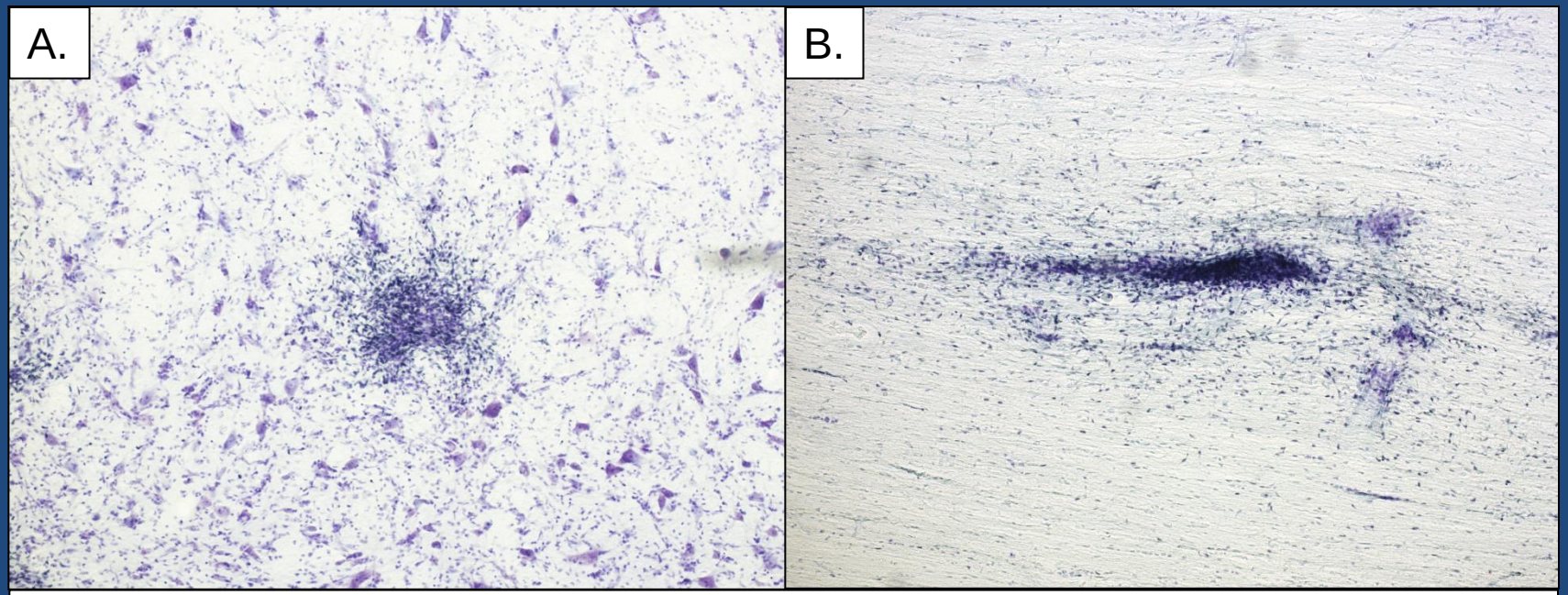
Not Damaged



Damaged

	Volume escalation (20 injections)			Number escalation (10 µL)	
Outcome measure	10 µL	25 µL	50 µL	30 injections	40 injections
Number of damaged injection sites	0 ± 0	0.8 ± 0.84	2 ± 1	0.6 ± 0.55	0.25 ± 0.5
* Note: values reported are mean ± standard deviation					

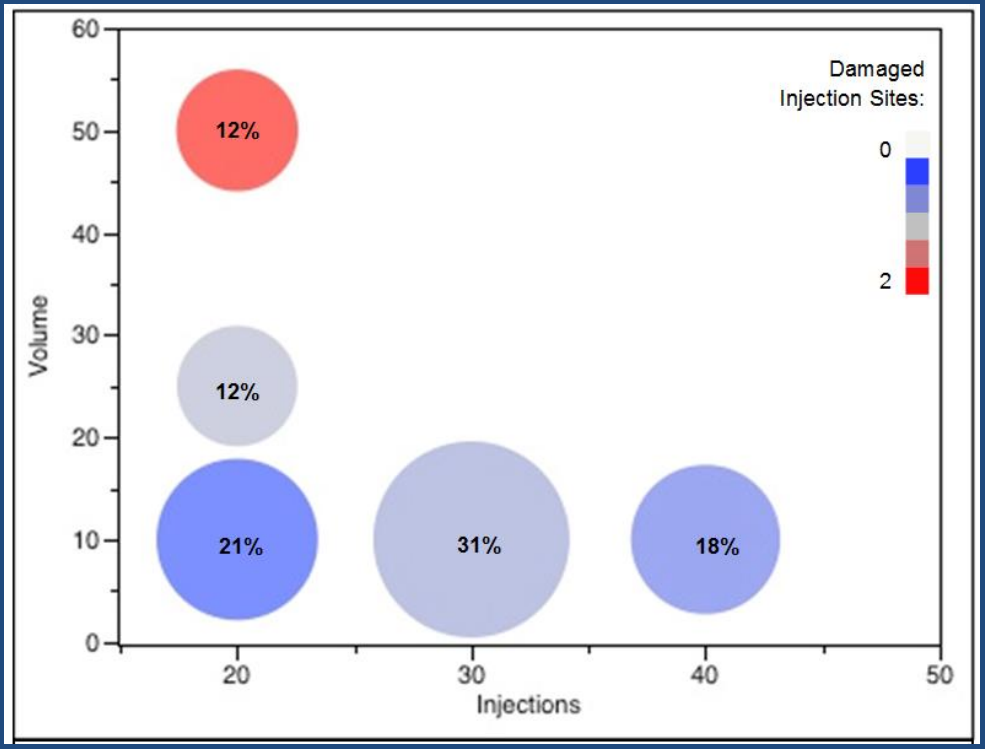
Histological Outcome – Migration Pattern



A) Grey matter (GM)10ul graft. B) White matter (WM) 10ul graft.

		Mean ± Std. Dev.	
Group	**n**	**Longitude**	**Latitude**
WM	6	1208.38 ± 502.63	283.58 ± 130.79
GM	5	391.66 ± 171.53	275.20 ± 94.84
	T-Test	**0.006**	**0.458**

Comparison of injection number and volume sized by engraftment percentage and colored by the number of damaged injection sites



As volume increases, the number of damaged injection sites increases and the engraftment percentage remains similar between groups (12-21%).
As number of injections increases, microscopic tissue damage stays relatively the same, but at 30 total injections the best engraftment percentage is achieved (31%).

The BLUER the BETTER - The BIGGER the BETTER